Customer Service or Technical Service:
In the U.S. Phone: 1-888-837-4436 (toll free)
OR E-Mail: productsupport@nanosphere.us
Outside the U.S.: Contact your local Nanosphere distributor
i www.e-labeling.eu/NAN023

Verigene® Enteric Pathogens Nucleic Acid Test (EP)

IVD

Rx Only

REF **20-005-023 (Test Kit) ● 20-012-023 (Amplification Kit)**

KEY-CODE **NAN023**

INTENDED USE

The Verigene® Enteric Pathogens Nucleic Acid Test (**EP**) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses, and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses:

- *Campylobacter* Group (composed of *C. coli*, *C. jejuni*, and *C. lari*)
- *Salmonella* species
- *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*)
- *Vibrio* Group (composed of *V. cholerae* and *V. parahaemolyticus*)
- *Yersinia enterocolitica*
- Norovirus GI/GII
- Rotavirus A

In addition, **EP** detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga toxins 1 and 2.

EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. **EP** also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

EP results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative **EP** results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

BACKGROUND INFORMATION AND CLINICAL UTILITY

Acute diarrhea caused by bacterial and viral infection represents a significant worldwide healthcare burden. The World Health Organization estimates that diarrhea causes or is a major contributor to approximately one-quarter of all post-neonatal childhood deaths.[1] The Centers for Disease Control and Prevention (CDC) estimate that 1.4 episodes of diarrhea occur per person per year in the USA.[2] Though most cases of diarrhea caused by enteric bacteria and viruses are self-resolving and not life-threatening, some can have serious implications.

Because clinical treatment decisions are often made based on the identity of the infecting pathogen, and in some cases the presence of an accompanying virulence gene, it is important to identify these targets quickly. Using multiplex molecular methods, it is possible to determine this information from a single nucleic acid test targeting the following analytes:

Shiga toxins 1 and 2: Shiga toxin-producing *Escherichia coli* (STEC), also referred to as enterohemorrhagic *E. coli* (EHEC) and verocytotoxic *E. coli* (VTEC), cause approximately 265,000 illnesses annually in the U.S., with more than 3,600 associated hospitalizations and 30 deaths.[3] A particularly virulent strain of STEC, O157:H7, accounts for about 75% of these illnesses.[4] Patients with STEC are at risk of developing a condition known as hemolytic uremic syndrome (HUS), a severe complication that can be fatal and is characterized by renal failure, hemolytic anemia, and thrombocytopenia.[3] Approximately 8% of the persons diagnosed with an O157:H7 STEC infection develops HUS.[5] Non-O157 STEC has also been responsible for illness in the US and throughout the world. The most commonly identified non-O157 serogroups responsible for illness in the U.S. include O26, O45, O103, O111, O118, O121, and O145.[3] If undiagnosed or under-reported incidences of STEC infections in the US are accounted for, an estimated 96,534 STEC O157 and 168,698 non-O157 infections occur annually.[3] The CDC recommends testing all stool cultures for shiga toxins and as of July 1, 2013, the Joint Commission mandates all member labs must test all stool cultures for O157 STEC using at least selective or differential media (QSA.04.06.01 EP 6).

Salmonella spp.: Infection with nontyphoidal *Salmonella spp*. causes diarrhea and fever. Approximately one million cases are known to occur annually in the U.S.[6] The *Salmonella* serotypes that cause a majority of human illness in the U.S. includes Typhimurium, Enteritidis, and Newport. Most cases of *Salmonella* infection, or salmonellosis, are typically self-resolving; however, patients at risk including children, elderly, and immunocompromised may require antimicrobial therapy to resolve symptoms.[7] Antimicrobial therapy can prolong the duration of non-typhoidal *Salmonella* and is only recommended for patients with the severe symptoms.

Campylobacter spp.: *Campylobacter* infection is the most common cause of bacterial gastroenteritis. Antibiotics are generally not prescribed unless symptoms are severe. Delaying treatment for several days while waiting for lab tests to confirm the presence of *C. jejuni* can reduce the effectiveness of the therapy.[8] The CDC reports that 14 cases of *Campylobacter* infection are diagnosed per 100,000 people in the United States annually, with many more cases going undiagnosed.[9] *Campylobacter* infections occur more frequently in summer months and the organism is more frequently recovered in young children over any other age group.[9] Infections with *Campylobacter* are more often than not self-resolving and do not require antimicrobials for treatment.

Yersinia enterocolitica: The disease yersiniosis is caused by *Y. enterocolitica*, which is an infection that can resemble Crohn's disease or appendicitis, with symptoms including diarrhea and fever. There is an estimated one culture-confirmed case of *Y. enterocolitica* infections per 100,000 persons in the United States each year.[10] Yersiniosis is self-limiting and does not generally require antibiotics. The CDC monitors the frequency of *Y. enterocolitica* infections through the foodborne disease active surveillance network (FoodNet).

Vibrio spp.: *Vibrio parahaemolyticus* is responsible for approximately 4,500 cases of illness in the United States annually.[11] *Vibrio parahaemolyticus* typically causes watery diarrhea along with nausea, vomiting, and abdominal cramps. Treatment is not usually required in a majority of cases of infection with *V. parahaemolyticus*. *V. cholerae* causes cholera, an infection in the small intestines resulting in profuse, watery diarrhea and vomiting. *V. cholerae* is relatively uncommon in developed countries, causing less than 100 cases annually in the U.S.[11] An

estimated 3-5 million cases of cholera occur each year worldwide, resulting in over 100,000 deaths.[11] Antibiotics can be used to shorten the duration of symptoms associated with cholera.

Shigella spp.: Infections with *Shigella* are known as shigellosis, with 14,000 cases reported in the United States each year. Patients infected with *Shigella*, a genus of gram-negative bacteria, often develop fever, stomach cramps, and bloody diarrhea. There are four serogroups of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. *S. sonnei* and *S. flexneri* are the most common causes of shigellosis in the United States. Although patients with mild *Shigella* infections usually recover without antibiotic treatment, antibiotics may be used to treat severe cases of shigellosis. Antidiarrheal agents can worsen illness and should therefore be avoided.[12]

Norovirus: Noroviruses are highly contagious, and cause on average 19-21 million cases of acute gastroenteritis each year.[13] Norovirus illnesses cost two billion dollars annually in the United States, ranking norovirus in the top five pathogens for enteric illnesses. Infection with Norovirus, a single-stranded non-enveloped RNA virus, causes nausea, vomiting, diarrhea, and abdominal pain. Norovirus infections constitute a major disease burden, which leads to high rates of hospitalization and mortality in children and the elderly.[14] Five distinct norovirus genogroups have been described (GI – GV), but human pathogens have been described only from genogroup I, genogroup II, and genogroup IV; however, genogroup IV norovirus is a rare cause of disease in the United States.[15]

Rotavirus: Globally, Rotavirus is the leading cause of severe diarrhea in infants and young children.[16] Rotavirus, a double-stranded non-enveloped RNA virus, is a cause of viral gastroenteritis and is most commonly seen in infants and young children. Symptoms include fever, vomiting, and watery diarrhea and may last upwards of 8 days after initial infection. Vaccination efforts have greatly reduced the incidence of rotavirus infection and the number of hospitalizations in the United States since 2006, when an estimated 60,000 children were hospitalized each year.[17] Antiviral drugs are ineffective against rotavirus, and the best treatment is management of dehydration.[18]

PRINCIPLES AND PROCEDURES OF VERIGENE EP AND THE VERIGENE SYSTEM

EP is performed using the Verigene System, which is a bench-top sample-to-result molecular diagnostics workstation consisting of two modules: the Verigene Processor *SP* and the Verigene Reader. The Verigene Processor *SP* automates the **EP** sample analysis steps including: (i) Specimen Preparation--Cell lysis and magnetic microparticle-based nucleic acid extraction from prepared stool specimens obtained from patients; (ii) Target Amplification--Multiplex PCR- and RT-PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons; (iii) Hybridization--amplicon hybridization to target specific capture DNA in a microarray format and mediator and gold-nanoparticle probe hybridization to captured amplicons. Silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that are imaged optically with high efficiency by the Verigene Reader. The Verigene Reader also serves as the user interface and central control unit for the Verigene System, storing and tracking information throughout the assay process.

The Verigene Processor *SP* utilizes single-use consumables to perform **EP**, including an Extraction Tray, Amplification Tray, and Test Cartridge. A separate Tip Holder Assembly contains two pipette tips that are used to transfer and mix reagents during the assay. The user tests a specimen by loading the single-use consumables into the Verigene Processor *SP*, pipetting the prepared specimen into the Extraction Tray, and initiating the protocol on the Verigene Reader by scanning or entering Test Cartridge ID and specimen information. Following assay completion, the user inserts the substrate slide portion of the Test Cartridge into the Verigene Reader for optical analysis and generation of test results.

MATERIALS PROVIDED

Verigene **EP** Test Kit (Catalog number 20-005-023)

- 20 Verigene **EP** Test Cartridges
  Each Test Cartridge comes preloaded with all required reaction reagents, including wash solutions, oligonucleotide probe solution and signal amplification solutions required to generate a test result. The Test Cartridges are labeled as: **EP; 20-006-023.**

- 20 Verigene **EP** Extraction Trays (with Tip Holder Assemblies)
  Each Extraction Tray comes preloaded with all required reagents, including lysis/binding buffer, wash solutions, and buffer solutions necessary to extract nucleic acids and generate a test result. The Extraction Trays are contained within a carrier labeled as: **EP; 20-009-023.**

- Verigene **EP** Stool Preparation Sample Kit
  Each Kit contains 20 tubes containing Verigene **EP** Stool Prep Buffer (SPB) and 20 swabs packaged in a resealable bag. The Kit is labeled as: **EP; 30-001-023.**

- 20 Verigene Sample Well Caps
  The Caps come packaged in strips of 5 Caps and are contained within a plastic bag. The bag is labeled as: **40-001-001.**

Verigene **EP** Test Amplification Kit (Catalog number 20-012-023)

- 20 Verigene **EP** Amplification Trays
  Each Amplification Tray comes preloaded with all required reagents, including enzymes and buffers necessary to amplify nucleic acids and generate a test result as well as an amplification tube. The Amplification Trays are contained within a carrier labeled as: **EP; 20-011-023.**

MATERIALS NEEDED BUT NOT PROVIDED

Instruments and Equipment

- Verigene Reader; Catalog number 10-0000-02
- Verigene Processor *SP*; Catalog number 10-0000-07
- 2-8°C Refrigerator
- ≤ -20°C Freezer
- ≤ -70°C Freezer (Optional)
- Micro-pipettors & filtered tips
- Vortex
- Table-top quick-spin Mini Centrifuge
- Decontamination wipes/spray or comparable sanitizer
- Biological Safety Cabinet (BSC)
- Verigene Extraction Tray Holder; Catalog number 421-00019-01 (Optional)

STORAGE, HANDLING, AND STABILITY

Table 1: Storage and Handling

Verigene EP Test Component	*Storage Conditions*	*Comments*
Stool Prep Buffer (SPB) Tubes & Swabs	20 – 30°C	Do not freeze.
Sample Well Caps		
Tip Holder Assemblies	2 – 30°C	Do not freeze.
Extraction Trays	2 – 8°C	Do not freeze.
Test Cartridges		
Amplification Trays	≤ - 20°C	Shipped frozen. Upon receipt, store frozen. Do not re-freeze after thawing.

VERIGENE DAILY MAINTENANCE

A. Work Area Preparation

Each day of testing and before and after sample preparation, prepare the testing work area by sanitizing the biological safety cabinet (BSC), countertops, vortex mixers, Mini Centrifuges, pipettes, and any other equipment used for sample processing with a lint-free decontaminating wipe.

B. Verigene System Cleaning

Prior to the start of testing each day, perform the following steps for each instrument being used for testing.

IMPORTANT: If there is liquid visible in the drawer assembly of the Verigene SP, or anything out of the ordinary is observed, do not proceed and immediately contact Nanosphere Technical Service.

1. While wearing fresh gloves, use a lint-free decontaminating wipe to thoroughly wipe the drawer assembly of the Verigene *SP*. For the Verigene Reader, wipe down the user interface screen and the door of the analysis compartment. It is not necessary to change gloves between instruments; however, do not use the same lint-free decontaminating wipe to clean different instruments.
2. If needed, dry the Verigene *SP* drawer assembly with a lint-free cloth prior to loading **EP** consumables.

METHODS

A. Specimen Collection & Storage

Inadequate or inappropriate specimen collection, storage, or transport may yield false-negative results. Due to the importance of specimen quality, training of personnel in the correct manner to perform specimen collection and handling is highly recommended.

1. Collect stool preserved in Cary-Blair medium by using the medium manufacturer's recommended collection procedure or collect unpreserved and unformed (liquid or soft) stool specimens and place as soon as possible into the Cary-Blair medium by using the medium manufacturer's recommended collection procedure.
2. It is recommended that Cary-Blair preserved specimens be stored refrigerated at 2-8°C until **EP** testing is completed (for up to 48 hours after collection). For repeat testing, prepare the specimen in a new Stool Prep Buffer as described in the Specimen Processing section (see Section B).

B. Fresh Cary-Blair Preserved Specimen Processing

1. Put on fresh gloves.
2. For each Cary-Blair preserved specimen to be tested, place one sterile flocked swab and one uncapped Stool Prep Buffer tube (place the cap to the side for recapping later) into a biological safety cabinet (BSC).
3. Wipe down the outside of the specimen vial with a lint-free decontaminating wipe.
4. Invert the vial containing the Cary-Blair preserved specimen twice and vortex the specimen for 5-10 seconds to ensure homogeneity.
5. To prepare the Stool Prep Buffer tube, dip the provided flocked swab into the Cary-Blair preserved specimen vial until the flocked tip is fully immersed in specimen. Once evenly coated, transfer the swab to the Stool Prep Buffer tube and break swab at the pre-formed scored breakpoint. Leave the swab in the Stool Prep Buffer tube and screw the cap finger tight on to Stool Prep Buffer tube.
6. Recap the original Cary-Blair preserved specimen container and set aside.
7. Repeat steps 1-6 for each specimen, changing gloves between each specimen.
8. Vortex each Stool Prep Buffer tube for 15-20 seconds.
9. Spin all prepared Stool Prep Buffer tubes in the Mini Centrifuge for 30-35 seconds.
10. Put on fresh gloves before continuing the procedure.

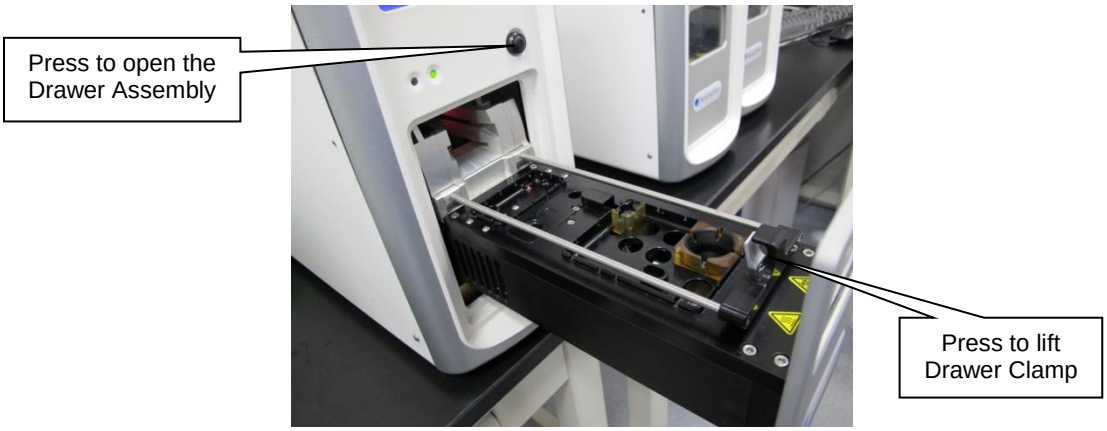
C. Verigene EP Test Procedure

Please refer to the Verigene System User's Manual for additional details on performing tests on the Verigene System as well as routine and daily maintenance.

1. Test set up—after Specimen Processing

    a) Remove an Extraction Tray, Tip Holder Assembly, and Test Cartridge from the refrigerator. Remove the Amplification Tray from the freezer and thaw at room temperature for a minimum of 10 minutes and begin the test run within 30 minutes (after removal from the freezer). Do not refreeze the Amplification Tray once it has been thawed.

b) The image below shows an empty Verigene Processor *SP*. Open the Drawer Assembly by pressing the black OPEN/CLOSE button located on the front of the Verigene Processor *SP*. Open the Drawer Clamp by pressing in the silver latch and lifting the Clamp prior to loading the consumables.



2. Loading the Extraction Tray

a) Prior to loading the Extraction Tray, thoroughly shake the Extraction Tray to resuspend the magnetic beads, which will have settled during storage. Check for complete resuspension by visually inspecting the well containing the beads. The well containing the magnetic beads is easily distinguished as the beads are black in color. Following adequate resuspension, gently tap the tray on the counter to ensure that the reagents settle to the bottom of each well.

Optional Cap Protocol: *If not using the Optional Cap Protocol, proceed to step 2b.*

i. Remove one cap from the strip of caps and place inside the BSC.

ii. *After shaking and tapping the Extraction Tray, place the Extraction Tray in the Extraction Tray Holder inside the BSC. (Refer to image below for visual of the Extraction Tray Holder).*

iii. *Pipette 200µL of the prepared sample into the bottom of the Sample Loading Well in the Extraction Tray. (Refer to the image below for Sample Loading Well location).*

Extraction Tray Holder

Extraction Tray

iv. *After sample loading, place the Sample Well Cap over the Sample Loading Well. Take precaution to handle only the edges of the Cap and firmly press down until the Cap is fully inserted into the Sample Loading Well.*

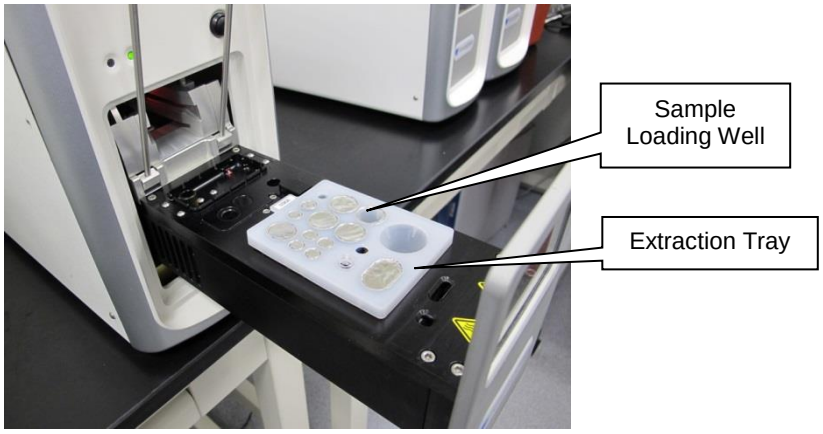
Sample Well Cap in Packaging

Pressing down on edges of cap

Extraction Tray with cap inserted

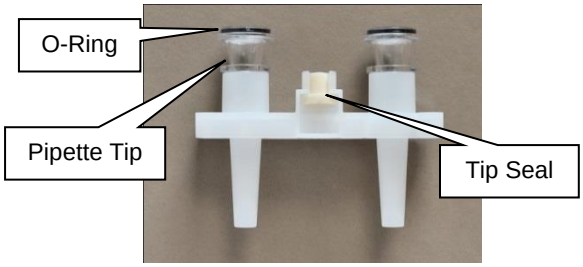
v. *Take the Extraction Tray out of the BSC and insert into the Extraction Tray Module on the Processor SP.*

b) The Extraction Tray can only be loaded in one location and orientation in the Drawer Assembly. When the Extraction Tray is loaded correctly, the Sample Loading Well is located at the right hand side of the Drawer Assembly. Place the Extraction Tray in the Drawer Assembly and press down on the corners of the tray to ensure it is level. The image below shows a properly loaded Extraction Tray.



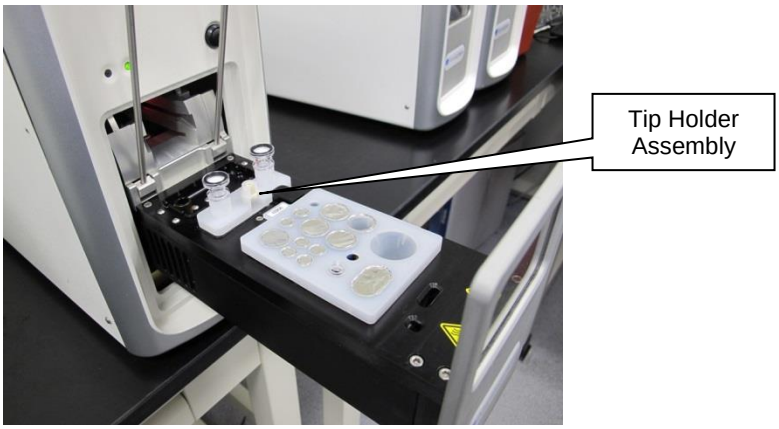
3. Load the Tip Holder Assembly

a) The Tip Holder Assembly is a plastic holder that contains two Pipette Tips and a rubber Tip Seal. Each Pipette Tip contains an O-ring on top.



b) Before using the Tip Holder Assembly, check the top of each Pipette Tip for the O-ring and confirm that the rubber Tip Seal is sitting straight and flush between the tips. If either is missing, replace with a new Tip Holder Assembly.

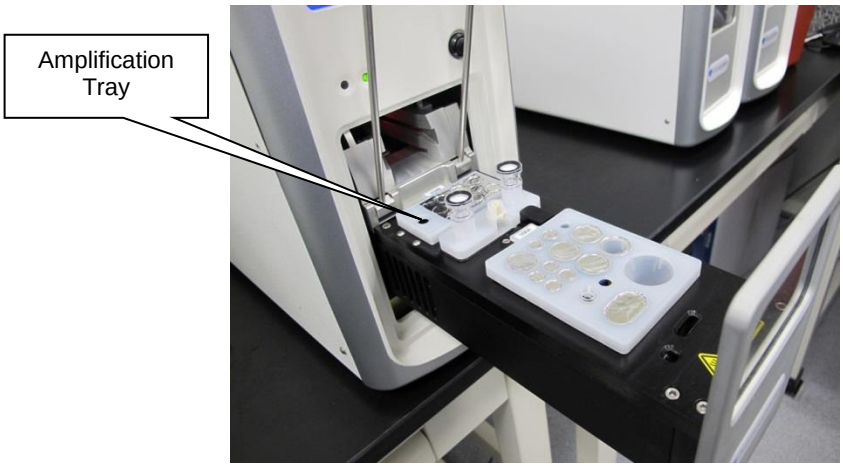
c) Insert the Tip Holder Assembly into the Drawer Assembly. The image below shows a properly loaded Tip Holder Assembly. The Tip Holder Assembly can only be loaded in one location and orientation in the Drawer Assembly. For orientation, there are two holes on the deck of the Drawer Assembly that fit each Pipette Tip and the opening to the Tip Seal should face away from the Processor *SP*.



4. Loading the Amplification Tray

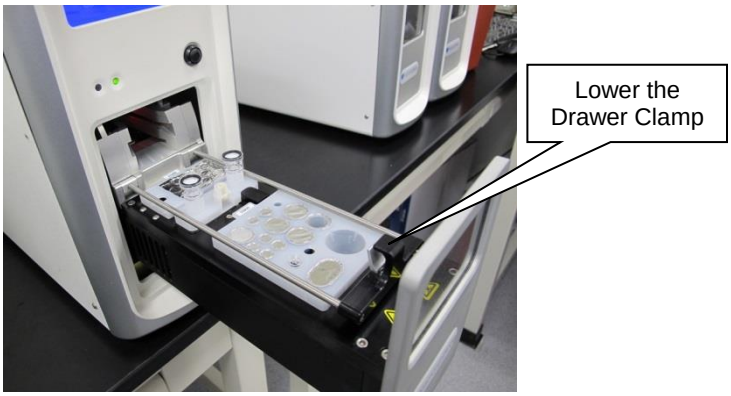
a) Remove the cap from the Amplification Tube from the thawed Amplification Tray and save the cap to re-cap the tube when processing is complete.

b) Insert the Amplification Tray into the Drawer Assembly. The image below shows a properly loaded Amplification Tray. The Amplification Tray can only be loaded in one location and orientation in the Drawer Assembly. When loaded properly, the tray sits flat.



c) Lower and latch the Drawer Clamp over the Trays while supporting the Drawer with the opposite hand. The image below shows a closed Drawer Clamp over properly loaded trays and Tip Holder Assembly. The Drawer Clamp will latch onto the Drawer Assembly when closed properly, and the user will be unable to lift the Drawer Clamp without pressing the sliver latch.

Note: If the Drawer Clamp is not latched properly, the Processor *SP* will display an error message on the Status Display when the user attempts to close the Drawer Assembly.



5. Ordering a Test

a) All tests must be ordered through the Verigene Reader. No test can be processed on the Verigene Processor *SP* without the user entering the Test Cartridge ID and Sample ID into the Verigene Reader.

i. Log into the Verigene Reader

ii. To start a new Session, proceed to the next step (iii). To order a test in a previously created session, select the desired Session from the drop down 'SESSION' menu, then proceed to step (v).

Note: Up to 60 Test Cartridges can be entered into a single session.

iii. From the Menu Bar, SESSION tab, select Start New Session where the Session Setup window will appear.

iv. Touch Session ID button and enter information by using the data entry keyboard. The Session ID can be any unique identifier in a format defined by the laboratory. The operator ID is automatically entered as the currently logged in "user."

v. Touch the Processing tab on the Navigation Bar at the bottom of the screen.

b) Enter the Test Cartridge ID by scanning the barcode using the barcode scanner attached to the Reader. The user may manually enter in the Test Cartridge ID by selecting MENU and 'Enter Barcode' and then keying in the Test Cartridge ID number with the Reader's keyboard.

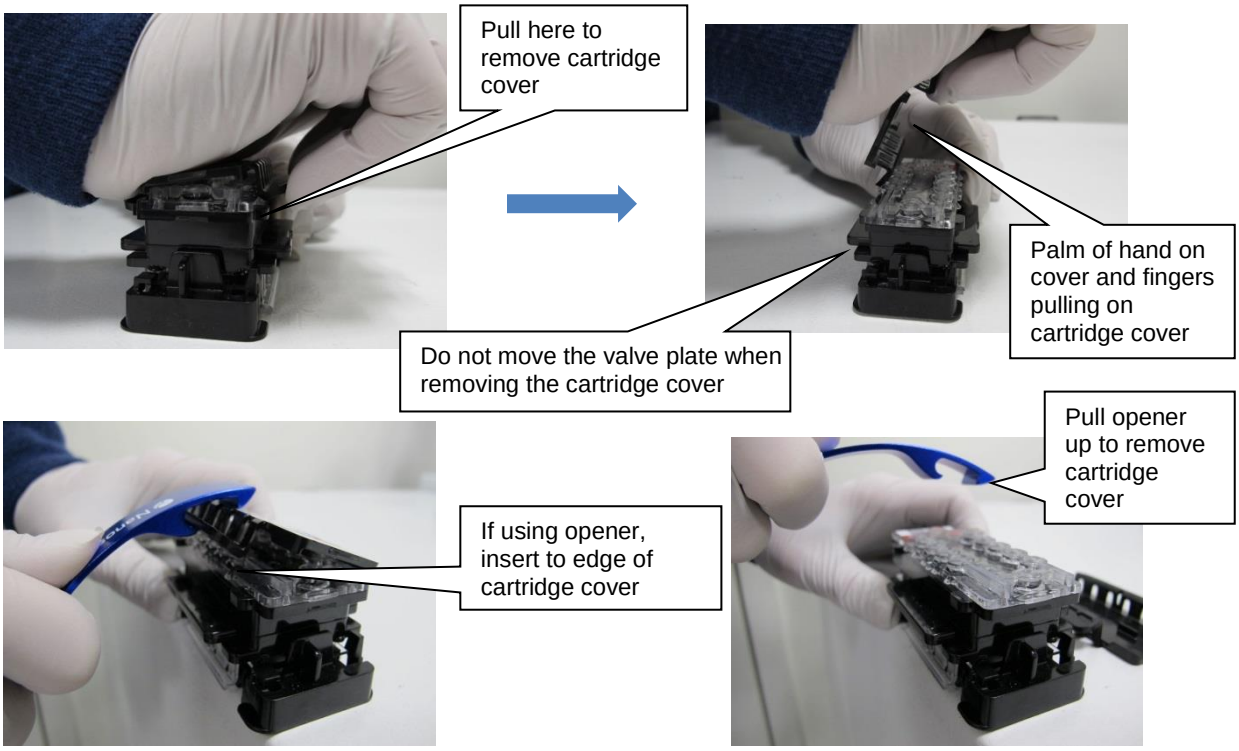
c) *(optional)* Scan the Test Cartridge Cover's 2D barcode using a barcode gun-style scanner to display the Test Cartridge's Reference Number, Expiration Date, and Lot Number on reports.

Note: The wand-style barcode scanner will not read 2D barcodes.

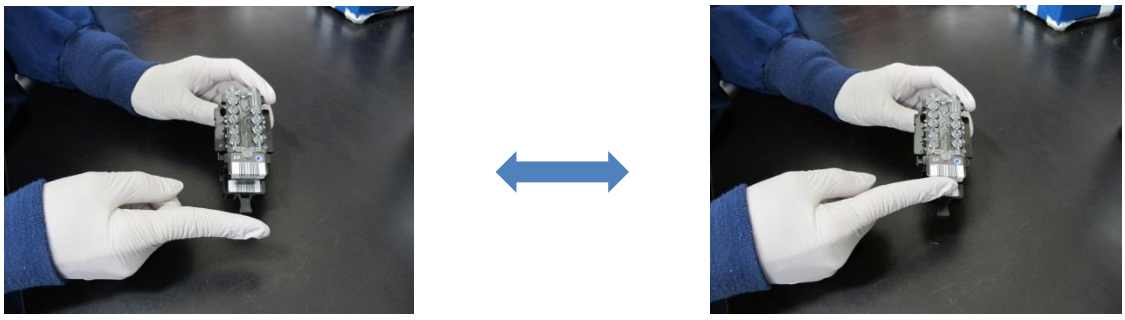
6. Load the Test Cartridge

a) Hold the Test Cartridge by the handle with one hand, using the other hand apply pressure with the palm of the hand and remove the Test Cartridge cover by bending the cover away and over the Reagent Pack edge (see illustration below). Alternatively, an opener may be used to remove the Test Cartridge cover. Ensure that the valve plate is not moved during cover removal.

Do not remove the Test Cartridge cover until immediately prior to inserting the Test Cartridge into the Processor *SP*.

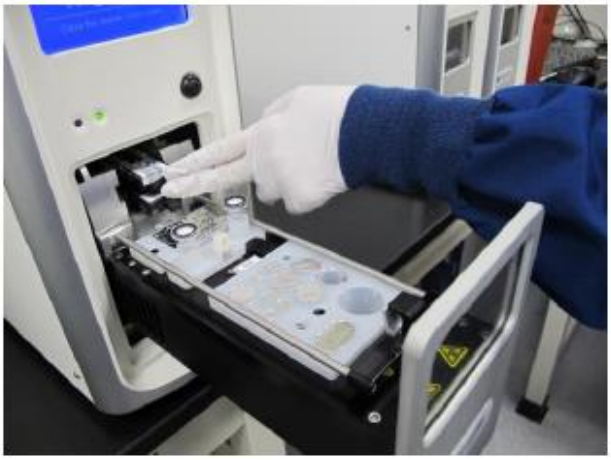

Pull here to remove cartridge cover
Palm of hand on cover and fingers pulling on cartridge cover
Do not move the valve plate when removing the cartridge cover
Pull opener up to remove cartridge cover
If using opener, insert to edge of cartridge cover

b) Settle the reagents in the Test Cartridge before loading into the Processor *SP*. The optimal method for setting the reagents is to hold the Test Cartridge's reagent container on the side opposite the handle and tap the barcode end of the Test Cartridge with your index finger. When tapping the Test Cartridge, allow the force of the tapping to move the Test Cartridge and your right hand. The tapping is more effective when the Test Cartridge is held in the air so that it moves slightly.



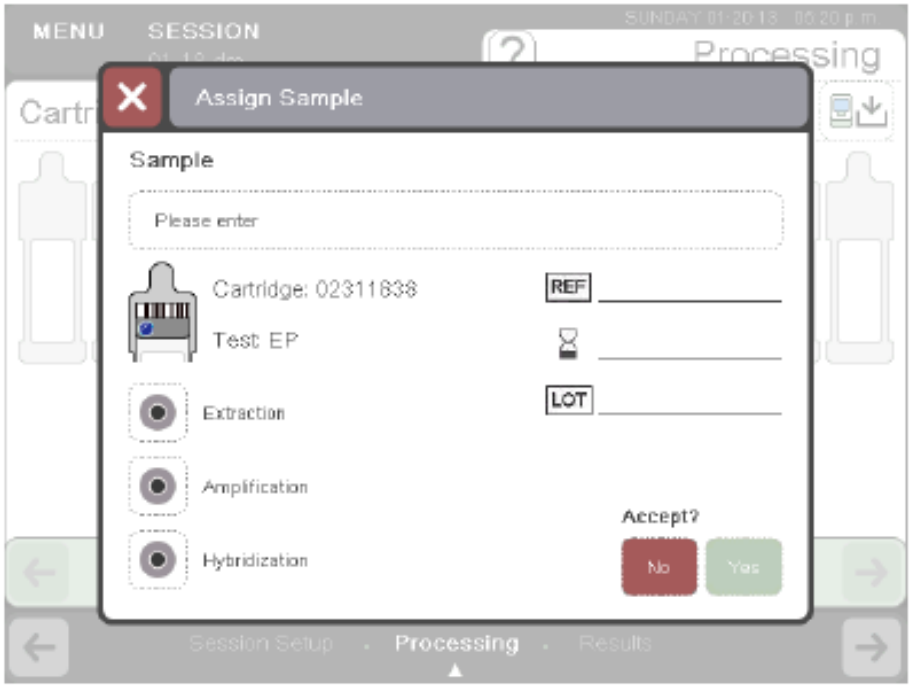
c) Insert the Test Cartridge into the Hybridization Module of the Processor *SP* until it reaches a stopping point. The image below shows the user loading a Test Cartridge into the Verigene Processor *SP*.

Note: If the Test Cartridge is not inserted properly, the Processor *SP* will display a message on the information screen when the user attempts to close the Drawer Assembly. If this occurs, remove the Test Cartridge from the Hybridization Module and re-insert the Test Cartridge.



7. Loading the Sample

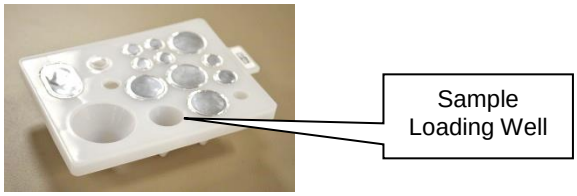
a) Enter the Sample ID by scanning or manually enter the Sample ID using the Reader's touch-screen keyboard. Press Yes to confirm the Sample ID. Ensure the Extraction, Amplification, and Hybridization options are selected (see image below).



b) In the subsequent dialogue box, select or de-select the bacterial, viral or toxin gene targets from the list to activate or de-activate results reporting for those targets. Press Yes to confirm. The Verigene Reader will automatically default to the selected targets for the next test run.

Note: Once a test run is started, results for de-selected targets cannot be retrieved.

c) (*If using optional Cap Protocol, sample has already been loaded. Skip to step 7d*) Pipette 200 µL of the prepared specimen in the Stool Prep Buffer into the Sample Loading Well of the Extraction Tray.



d) Close the Drawer Assembly by pressing the OPEN/CLOSE button on the Processor *SP*. The Processor *SP* will automatically verify that each consumable is properly loaded and being sample processing.

e) Confirm countdown has started on the Processor *SP* display screen before leaving the area.

f) In order to set up additional tests on other Processor *SP* instruments, follow the same procedure. To avoid contamination and sample mix-ups, set up one test at a time, change gloves after handling a sample, and decontaminate pipettes and sample tubes.

Note: Store the original Cary-Blair specimen at room temperature until completion of Verigene System testing.

8. Upon Completion of a Test Run

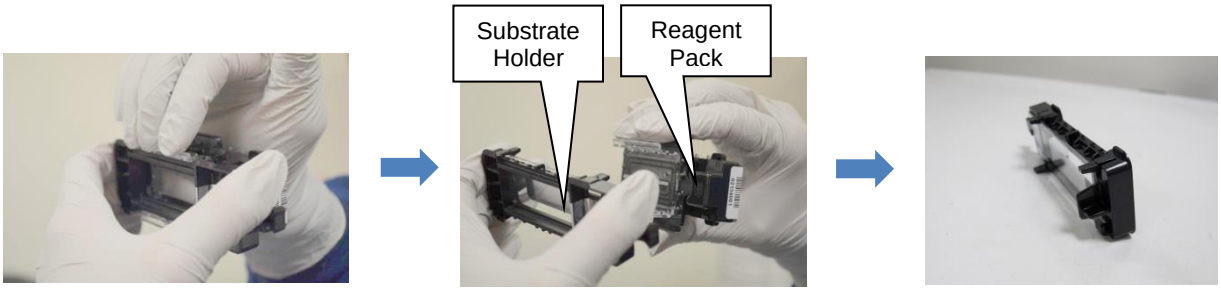
a) The Verigene Reader will generate a ring to notify the user when the test is completed and the Processor *SP* will display a message indication “Procedure Complete. Ready to Open Drawer.” The Test Cartridge should be removed from the Processor *SP* upon completion of the test.

b) Open the Drawer Assembly by pressing the OPEN/CLOSE button.

c) Cap the Amplification Tube for disposal.

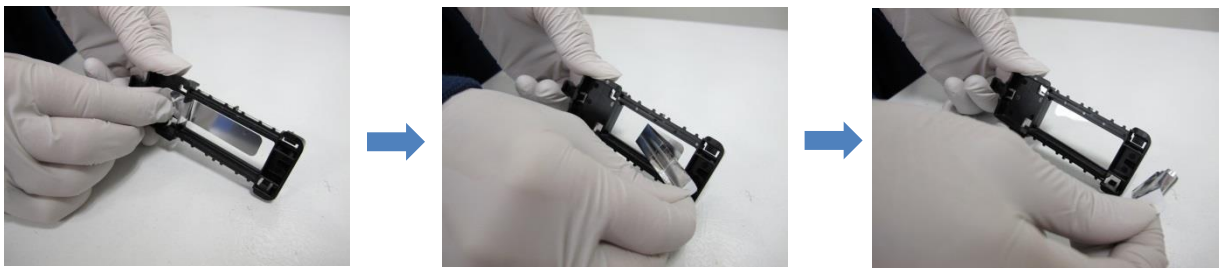
d) Remove the Test Cartridge and immediately orient to its side.

e) While keeping the Test Cartridge on its side, separate the Reagent Pack and keep the Test Substrate on its side for 30-60 seconds after removal, as illustrated below to allow the final rinse to dry away from the analysis area.



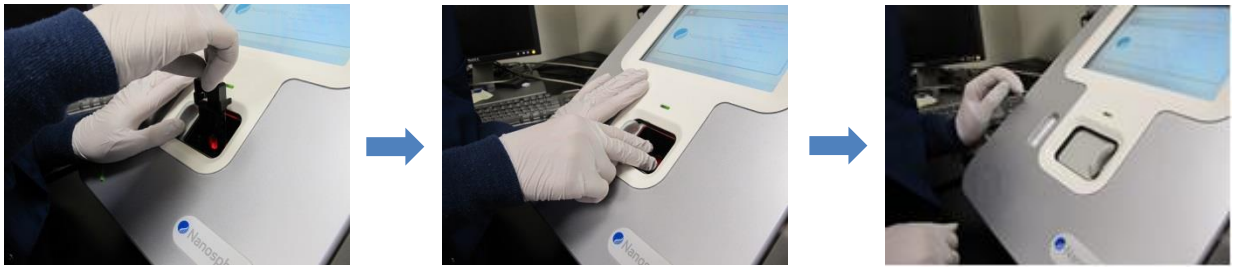
9. Analyze Results

a) Remove the protective tape from the back of the slide in the Substrate Holder.



b) Use the Reader's barcode scanner to read the barcode on the Substrate Holder. When the barcode is accepted, a prompt to load the Substrate Holder into the Reader will be displayed.

c) Immediately insert the Substrate Holder into the Reader.



d) When the load substrate prompt occurs, it will only display for 20 seconds. The analysis will only start if the Substrate is loaded during the animated prompt.

e) To properly insert the Substrate Holder into the Reader, hold the Substrate Holder by the handle with the barcode facing away from you. Next, insert the Substrate Holder into the Substrate Compartment. The Substrate Compartment is designed to place the Substrate Holder in the correct position. Do not force the Substrate Holder in, but do insert it into the Substrate Compartment as far as it will go comfortably. Close the door of the Substrate Compartment.

f) The analysis will automatically begin. A small camera icon will appear on the Reader to indicate that analysis has begun.

g) Once the analysis is completed by the Reader, the camera icon will be replaced with an upward facing arrow and the Reader rings.

h) Confirm that a result other than 'No Call – NO GRID' has been generated by touching the Substrate icon for the test. A Substrate producing a 'No Call – NO GRID' result should be reanalyzed.

i) Once the scan is complete, dispose of the used Test Substrate and the used Reagent Pack.

j) To access the remaining used consumables, raise the Drawer Clamp, remove and dispose the used Extraction and Amplification Trays and the Tip Holder Assembly.

10. Printing Results

a) Touch the Substrate icon in the Session's Processing screen. A window displaying the results will open. Touch the 'Print' option on this screen to print a Detail Report.

b) A Summary Report is available by moving to the Results screen of the Session on the bottom Navigation Bar; go to MENU then select 'Print Summary.' The Summary Report will provide the results for all tests processed within the current Session.

c) Detail Reports can also be viewed and printed from the Results window. First, select the desired test from the list, go to MENU and then touch 'Print Detail.'

INTERPRETATION OF RESULTS

EP provides a qualitative result for the presence (Detected) or absence (Not Detected) of the **EP** target genes. The image analysis of the Test Substrate provides light signal intensities from the target-specific capture spots as well as the negative control, background, and imaging control spots. The mean signal intensity of a target is compared to the assay's signal detection threshold to make a call. **Table 2** lists the possible test results generated by **EP** representing identification of bacterial, viral, and/or genetic virulence marker nucleic acid sequences/targets. Their presence is verified before a valid result is provided as described below.

Table 2: Calls for Valid Tests

Organism/Gene	***Target Genes***	***Test Result Reported as "Detected"***		
		Genus/Group	***Species***	***Toxin Gene***
Campylobacter Group[a]	*fusA*	*Campylobacter*	-	-
Salmonella species	*rpoD*	*Salmonella*	-	-
Shigella species[b]	*ipaH*	*Shigella*	-	-
Vibrio Group[c]	*rfbL, trkH, & tnaA*	*Vibrio*	-	-
Yersinia enterocolitica	*recN*	-	*Yersinia enterocolitica*	-
Shiga Toxin 1	*stx1*	-	-	*Shiga Toxin 1*
Shiga Toxin 2	*stx2*	-	-	*Shiga Toxin 2*
Norovirus GI/GII	*ORF1-ORF2 junction region*	Norovirus	-	-
Rotavirus A	*nsp5*	Rotavirus	-	-
All Analytes "Not Detected"	-	-	-	-

[a] *C. coli*, *C. jejuni*, and *C. lari*
[b] *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*
[c] *V. cholerae* and *V. parahaemolyticus*

Error calls related to an invalid test are listed in the **Table 3** below, together with the appropriate recourse that should be taken by the user.

Table 3: Invalid Calls and Recourse

Call	***Reason***	***Recourse****
No Call – INT CTL 1	Internal Control 1 not detected indicating target hybridization issue	Repeat **EP**
No Call – INT CTL 2	Internal Control 2 not detected indicating lysis, extraction, or amplification issue	Repeat **EP**
No Call – INT CTL	INT CTL 1 and INT CTL 2 not detected indicating lysis, extraction, amplification, or target hybridization issue	Repeat **EP**
No Call – NO GRID	Reader unable to image Test Substrate	Ensure Test Substrate is seated properly in the substrate holder. Repeat image analysis by selecting 'Menu' and 'Enter Barcode' and then scanning the Substrate barcode. If the No-Call persists, repeat **EP** from original stool specimen
No Call – VARIATION	Reader unable to obtain test result because of high variability in the target-specific signals	Repeat **EP**
No Call – BKGD		
No Call – NEG CTL		
Processing Error	Pre-Analysis Error--Internal checks within the Processor *SP* detected an unexpected event	Power cycle the Processor *SP* and repeat **EP**

* Repeat tests should be from the Cary-Blair preserved stool specimen into a new Stool Prep Buffer tube.

QUALITY CONTROL

Quality control, as a component of an overall quality assurance program, consists of tests and procedures for monitoring and evaluating the analytical performance of a measurement system to ensure the reliability of patient test results.

Verigene System

The Verigene System uses a series of automated on-line quality measurements to monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps each time a test is performed. A series of automated on-line procedural checks guide the user through the testing process each time a test is performed. The **EP** barcode and specimen information are linked upon entry into the Verigene Reader to help prevent misreporting of results.

Assay Controls

EP is a 'specimen-to-result' detection system wherein nucleic acids are isolated from unformed stool specimen and specific detection is performed on an oligonucleotide array housed within the Test Cartridge. To prevent reagent dispensing errors, all reagents are prepackaged in single-use disposables, including Stool Prep Buffer Tubes, reagent trays, and cartridges. Several layers of controls built into **EP** ensure that failures at any step within **the test** are identified during the procedure or in the end-point image analysis of the Test Cartridge.

Internal Processing Controls

An artificial DNA construct serves as a target hybridization control and is referred to as the Internal Processing Control 1 (INT CTL 1). If the INT CTL1 is not valid, a 'No Call – INT CTL 1' result will be obtained and the test should be repeated.

MS2 Phage serves as a specimen isolation and amplification control and is referred to as the Internal Processing Control 2 (INT CTL 2). This control is automatically added by the Verigene *SP* to each specimen prior to the extraction step. If the process control is not valid a 'No Call – INT CTL 2' result will be obtained and the test should be repeated.

If both INT CTL 1 and INT CTL 2 are not detected, a 'No Call – INT CTL' result is generated. These internal controls are not utilized for the detection of positive samples.

Additional positive controls are immobilized on the Test Slide. These are used to determine that hybridization was performed correctly. The **EP** algorithm requires that these controls be valid before decisions regarding the absence of any other target on the panel can be determined. If these controls are not detected a No Call result will be obtained and the test should be repeated.

Table 4: Internal Processing Controls

Control	***Description***	***Function***
Internal Process Control (INT CTL 1)	Artificial DNA construct with detection oligonucleotides	Controls for target hybridization-related issues
Internal Process Control (INT CTL 2)	Intact MS2 Phage along with primers and detection oligonucleotides. Added to each test specimen	Controls for lysis, extraction, and target amplification

External Controls

Regardless of the choice of quality control materials, all external quality control requirements and testing should be performed in conformance with local, state, and federal regulations or accreditation organizations as applicable and should follow the user's laboratory's standard quality control procedures.

TROUBLESHOOTING

Refer to the Troubleshooting section of the Verigene System User's Manual.

LIMITATIONS

- A trained health care professional should interpret assay results together with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- In rare instances, *Campylobacter insulaenigrae* may yield a false positive "Campylobacter detected" result.
- The following 15 species of *Vibrio*, each of which are NOT associated with infections in humans and therefore unlikely to be encountered in human stool, were shown NOT to be detected by **EP** based upon in silico analysis only: *V. anguillarum, V. brasiliensis, V. coralliilyticus, V. crassostreae, V. cyclitrophicus, V. ichthyoenteri, V. kanaloae, V. nigripulchritudo, V. ordalii, V. orientalis, V. rotiferianus, V. rumoiensis, V. scophthalmi, V. splendidus,* and *V. tasmaniensis*.
- The following 27 species of Vibrio, each of which are NOT associated with infections in humans and therefore unlikely to be encountered in human stool, were not evaluated for exclusivity by empirical testing or in silico analysis due to a lack of genome sequence information: *V. aerogenes, V. aestuarianus, V. chagasii, V. diabolicus, V. diazotrophicus, V. ezurae, V. fortis, V. gallicus, V. gazogenes, V. gigantis, V. halioticoli, V. hepatarius, V. hispanicus, V. litoralis, V. mediterranei, V. mytili, V. natriegens, V. navarrensis, V. neonatus, V. nereis, V. pacini, V. pectenicida, V. pomeroyi, V. proteolyticus, V. ruber, V. superstes,* and *V. xuii.*
- **EP** is expected to be inclusive to most strains of the Norovirus GI, GII, and GIV genotypes known to cause disease in humans based on empirical testing and supplemented by *in silico* analysis. However, due to the high genetic diversity within Noroviruses, some strains may not be detected or may be detected with reduced sensitivity by **EP**. Refer to the Analytical Sensitivity (Inclusivity) section and Table 16 for details.
- **EP** inclusivity to Norovirus strains GII.9, GII.14, and GIV.1 was evaluated by *in silico* analysis only. Rare Norovirus genotypes GII.6 and GII.13 were determined to either be detected at reduced sensitivity or predicted to not be detected by **EP** based on *in silico* analysis. For GII.8, **EP** inclusivity is unknown as, in the absence of sequence information, *in silico* analysis could not be performed.
- Norovirus GII.11 is not expected to be detected by EP based on *in silico* analysis.
- Norovirus GIII, GIV.2, and GV are not expected to be detected based on *in silico* analysis.
- **EP** inclusivity to Rotavirus A genotypes, G4, G5, G10, G11, and G15 was evaluated based on *in silico* analysis only. Inclusivity of **EP** to Rotavirus A genotypes G7, G21, and G24 is unknown; representative strains were not available for empirical testing and in the absence of sequence information, *in silico* analysis could not be performed.
- Rotavirus genogroups B, D, and NADRV are not expected to be detected by **EP** based on *in silico* analysis. In addition, Rotavirus genogroup C strains are not expected to be detected by **EP**, with the exception of porcine strains within this genogroup.

WARNING AND PRECAUTIONS – GENERAL

- **EP** is for *in vitro* diagnostic use.
- ***Caution: Federal law restricts this device to sale by or on the order of a physician or to a clinical laboratory***.
- Never use any Tips, Trays, Tubes, or Test Cartridges that have been broken, cracked, punctured, previously used or visibly damaged; using damaged material may lead to No Call or false results.
- Handle supplies, reagents, and kits with powder-free gloves at all times to avoid contamination and change gloves between removal of used disposables and loading of new disposables.
- Handle specimens carefully. Open one tube or specimen at a time to prevent specimen contamination.
- Biological specimens such as stool, tissues, body fluids, and blood of humans are potentially infectious. When handling and/or transporting human specimens, follow all applicable regulations mandated by local, state/provincial, and federal agencies for the handling/transport of etiologic agents.
- National, state, and local public health authorities have published guidelines for notification of reportable diseases in their jurisdictions including *Salmonella*, *Shigella*, *Vibrio* and Shiga-like Toxin producing *E. coli* (STEC) stx1/stx2 to determine necessary measures for verification of results to identify and trace

outbreaks. Refer to The CDC's Nationally Notifiable Disease Surveillance System (http://wwwn.cdc.gov/nndss/) for additional information and resources. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.

WARNINGS AND PRECAUTIONS – INSTRUMENT

A. General Instrument Safety

WARNING: Use this product only as specified in this document. Using this instrument in a manner not specified by Nanosphere may result in personal injury or damage to the instrument. Anyone who operates the instrument must have:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDS).

B. Electrical Shock Hazard

WARNING: Severe electrical shock can result from operating the instrument without its instrument covers or back panels in place. Do not remove instrument covers or panels. High-voltage contacts are exposed when instrument covers or panels are removed from the instrument. If service is required, contact Nanosphere Technical Support at 1-888-837-4436.

C. Maintenance of the Verigene Reader and Verigene Processor *SP*

For routine and daily maintenance instructions, please refer to the Verigene System User's Manual.

WARNGINGS AND PRECAUTIONS – REAGENTS AND TEST CARTRIDGES

A. Material Safety Data Sheets

- Material Safety Data Sheets (MSDS) for the Test Cartridge, Amplification Tray, and Extraction Tray are available at www.e-labeling.eu, www.nanosphere.us, or upon request from Nanosphere, Inc.

B. Toxicity of Reagents

- Exposure to chemicals sealed inside the Test Cartridge is hazardous in case of skin contact and of ingestion. Protective disposable gloves, laboratory coats, and eye protection should be worn when handling specimens, Extraction Trays, Amplification Trays, and Verigene Test Cartridges.
- See Material Safety Data Sheets (MSDS) for toxicity information.

C. Waste Disposal

- The Amplification Tray contains amplification reagents and the internal controls. Dispose the Amplification Tray in accordance with national, state, and local regulations.
- The Extraction Tray contains residual nucleic acids, extraction reagents, and residual sample. It also contains a residual volume of the sample buffer which contains formamide, a teratogen. Dispose the Extraction Tray and Stool Prep Buffer tube in accordance with national, state, and local regulations.

- All of the waste reagents, including the purified nucleic acids, are contained within the Test Cartridge. There is a very small amount of residual formamide (≤1% v/v). Dispose the Test Cartridge in accordance with national, state, and local regulations.

EXPECTED VALUES

Prevalence

In the **EP** Methods Comparison study, 1328 prospectively collected fresh and frozen specimens were obtained from seven medium- to large-sized healthcare institutions geographically distributed across the United States. The number and percentage of positive cases (positivity rate) determined by **EP** stratified by geographic region for each of the organisms detected by the test are presented in **Table 5**. Overall, **EP** detected at least one target in 11.2% (149/1328) of prospectively collected specimens. In routine practice, prevalence rates may vary depending on the institution, geographical location, and patient population.

Table 5: Prevalence of Organisms Detected by **EP** – Clinical Study Observations

		*US Geographic Region/Division**					Total
Target	**Region**	*Midwest*		*South*	*Northeast*	*West*	
	Division	*West North Central*	*East North Central*	*W. South Central*	*Middle Atlantic*	*Pacific*	
	State	*MO*	*WI*	*TX*	*NY*	*CA*	
	Total n	10	196	119	232	771	1328
Campylobacter	POS n	0	7	5	5	22	39
	% Prev.	-	3.6	4.2	2.2	2.8	2.9
Salmonella	POS n	1	2	1	6	18	28
	% Prev.	10.0	1.0	0.8	2.6	2.3	2.1
Shigella	POS n	1	0	4	1	13	19
	% Prev.	10.0	-	2.5	0.4	1.7	1.4
Vibrio	POS n	0	0	0	0	2	2
	% Prev.	-	-	-	-	0.3	0.2
Y. enterocolitica	POS n	0	0	0	0	0	0
	% Prev.	-	-	-	-	-	-
Stx1	POS n	0	1	1	1	4	7
	% Prev.	-	0.5	0.8	0.4	0.5	0.5
Stx2	POS n	0	1	1	1	5	8
	% Prev.	-	0.5	0.8	0.4	0.6	0.6
Norovirus	POS n	0	2	2	4	34	42
	% Prev	-	1.0	1.7	1.7	4.5	3.2
Rotavirus	POS n	0	0	0	0	3	3
	% Prev	-	-	-	-	0.4	0.2

**Geographic Areas Reference Manual* (US Census Bureau).Chapter 6 https://www.census.gov/geo/reference/pdfs/GARM/Ch6GARM.pdf. Webpage last revised: 9/16/2013

PERFORMANCE CHARACTERISTICS

The results of the analytical and clinical studies conducted to establish the performance characteristics of **EP** are provided below.

A. Clinical Performance

A method comparison study was conducted at multiple external clinical study sites to evaluate the performance of **EP.** Bacterial results were compared to reference culture, followed by bacterial biochemical identification. Stx1 and Stx 2 specimens were identified using enriched culture and EIA, with positive specimens typed for bacterial virulence markers by PCR amplification followed by confirmatory bi-directional sequencing. **EP** viral test results were evaluated by comparison to a composite of three RT-PCR methods and bi-directional sequencing (BDS). Subjects included individuals whose routine care called for enteric pathogen testing.

There were 1940 evaluable specimens enrolled in the clinical trial; 78 specimens resulted in an initial **EP** No Call for a No Call rate of 4.0% (78/1940 specimens). Sixteen (16) specimens incurred an initial Pre-Analysis Error (PAE) and three (3) specimens incurred a PAE upon repeat, yielding a Pre-Analysis Error rate of 0.9% (19/2063 tests run) for a total initial valid test rate of 95.1%. Of the 78 initial No Calls, 52 yielded a valid test result upon retesting and of the 16 initial PAEs, thirteen (13) specimens yielded a valid call upon repeat. The final No Call rate was 1.3% (26/1940 specimens) and the final Pre-Analysis Error rate was 0.1% (3/2063 tests run) for a total final valid test rate of 98.7%. The twenty-six (26) specimens which yielded a final No Call result were not included in the valid dataset utilized in the comparative test result data analysis. Therefore, 98.7% (1940/1966) of the valid specimens were analyzed in this clinical evaluation to establish clinical performance of the test; 1294 of which were prospectively collected fresh specimens, 34 of which were prospectively collected frozen specimens, 203 of which were selected frozen specimens, and 409 of which were simulated frozen specimens.

The clinical performance of **EP** is summarized below in **Table 6** for the five bacterial targets (n=1940), in **Table 7** for the Stx Combined targets, **Table 8** for the Stx1 and Stx2 targets (n=1940), and **Table 9** for the two viral targets (n=1942).

Table 10 contains additional genus/group-level specific **EP** performance data stratified by individual species within each genus; i.e.; *Campylobacter Group., Salmonella spp., Shigella spp.,* and *Vibrio Group.*

In total, there were 25 mixed specimens that were detected by **EP,** and 11 mixed specimens detected by the reference comparator methods. **Table 11** lists the distinct mixed specimen combinations detected by **EP** in the clinical study and **Table 12** lists the distinct mixed specimen combinations detected by the reference/comparator methods, which were all detected by **EP.**

Table 6: Summary of Bacterial Target Clinical Test Performance (n=1940) Compared to Reference Methods (Culture and Conventional Biochemical and Automated Phenotypic Identification)

	Specimen Type			*n*	*% Agreement (95% CI)*	
					Positive	***Negative***
Campylobacter spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	90.9% 20/22[a] (79.8-98.9)	98.7% 1255/1272[d,q] (97.9-99.2)
			Frozen	34	100% 2/2 (15.8-100)	100% 32/32 (89.1-100)
		Selected		203	97.5% 39/40[c] (86.8-99.9)	99.4% 162/163[e] (96.6-100)
	Simulated			409	98.5% 67/68[b] (92.1-100)	100% 341/341 (98.9-100)
***Salmonella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	86.4% 19/22[f,r] (65.1-97.1)	99.4% 1265/1272[h] (98.9-99.8)
			Frozen	34	100% 1/1 (2.5-100)	97.0% 32/33[i] (84.2-99.9)
		Selected		203	98.3% 58/59[g] (90.9-100)	99.3% 143/144[j] ((96.2-100)
	Simulated			409	100% 67/67 (94.6-100)	100% 342/342 (98.9-100)
***Shigella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3[k,r] (9.4-99.2)	98.8% 1275/1291[l] (98.0-99.3)
			Frozen	34	-	97.1% 33/34[m] (84.7-99.9)
		Selected		203	100% 8/8 (63.1-100)	99.5% 194/195[n] (97.2-100)
	Simulated			409	100% 50/50 (92.9-100)	100% 359/359 (99.0-100)
***Vibrio* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 1/1 (2.5-100)	100% 1293/1293 (99.7-100)
			Frozen	34	100% 1/1 (2.5-100)	100% 33/33 (89.4-100)
		Selected		203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)
	Simulated			409	91.1% 51/56[o] (80.4-97.0)	99.7% 352/353[p] (98.4-100)
Y. enterocolitica	Clinical Specimens	Prospectively Collected	Fresh	1294	-	100% 1294/1294 (99.7-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)
	Simulated			409	100% 59/59 (93.9-100)	100% 350/350 (99.0-100)

	No.	Type	EP Test Result	Reference Method Result(s)	PCR Amp/BDS Results
a.	1	Fresh	Not Detected	*C. jejuni subsp. jejuni*	*Positive for Campylobacter jejuni*
	2	Fresh	Not Detected	*C. jejuni subsp. jejuni & Proteus spp.*	*Negative for Campylobacter spp.*
b.	1	Simulated (2X)	Not Detected	*C. lari*	*Positive for Campylobacter lari*
c.	1	Select	Not Detected	*Campylobacter*	*Low-Level Positive for Campylobacter jejuni (at LoD; Negative upon repeat))*
d.	1	Fresh	Campylobacter	Negative	*Positive for Campylobacter coli*
	2	Fresh	Campylobacter	*M. morganii subsp. morganii & N. cinerea*	*Positive for Campylobacter jejuni*
	3	Fresh	Campylobacter	*P. aeruginosa*	*Positive for Campylobacter jejuni*
	4	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	5	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	6	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	7	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	8	Fresh	Campylobacter	*M. morganii subsp. morganii*	*Positive for Campylobacter jejuni.*
	9	Fresh	Campylobacter	*C. braakii & E. cloacae subsp dissolvens & N. cinerea*	*Positive for Campylobacter jejuni*
	10	Fresh	Campylobacter	Negative	*Positive for Campylobacter spp.*
	11	Fresh	Campylobacter	Negative	*Positive for Campylobacter spp.*
	12	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	13	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	14	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	15	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	16	Fresh	Campylobacter	*Proteus spp.*	*Positive for Campylobacter coli*
	17	Fresh	Campylobacter	*M. morganii subsp. morganii*	*Positive for Campylobacter jejuni*
e	1	Select	Campylobacter and Salmonella	*Salmonella*	*Positive for Campylobacter jejuni and Salmonella enterica*
f.	1	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
	2	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
	3	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
g.	1	Select	Not Detected	*Salmonella spp.*	*Low-Level Positive for Salmonella enterica (at LoD; Negative upon repeat)*
h.	1	Fresh	Salmonella	Negative	*Negative for Salmonella spp.*
	2	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
	3	Fresh	Salmonella	*E. coli*	*Positive for Salmonella enterica*
	4	Fresh	Salmonella	*C. freundii & Proteus spp.*	*Negative for Salmonella spp.*
	5	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
	6	Fresh	Salmonella	*P. alcalifaciens*	*Negative for Salmonella spp.*
	7	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
i.	1	Frozen	Salmonella	*Proteus spp.*	*Positive for Salmonella enterica*
j.	1	Select	Salmonella	Campylobacter	*Positive for Campylobacter jejuni*
k.	1	Fresh	Not Detected	*Shigella spp.*	*Positive for Shigella/EIEC*
l.	1	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	2	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	3	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	5	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	6	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	7	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	8	Fresh	Shigella	*A. hydrophila/cavieae & P. putida*	*Positive for Shigella/EIEC*
	9	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	10	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	11	Fresh	Shigella	*E. coli*	*Positive for Shigella/EIEC*
	12	Fresh	Shigella	Negative	*Not performed*
	13	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	14	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	15	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	16	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
m.	1	Frozen	Shigella	*P. rettgeri*	*Positive for Shigella/EIEC*
n.	1	Select	Shigella	Shiga toxin	*Positive for Shiga toxin 1*
o.	1	Simulated (2X)	Not Detected	*Vibrio parahaemolyticus*	*Positive for V. parahaemolyticus*
	2	Simulated (2X)	Not Detected	*Vibrio cholerae*	*Positive for V. cholera*
	3	Simulated (40X)	Not Detected	*Vibrio cholerae*	*Negative for V. cholera*
	4	Simulated (40X)	Not Detected	*Vibrio parahaemolyticus*	*Negative for V. parahaemolyticus*
	5	Simulated (40X)	Not Detected	*Vibrio cholerae*	*Positive for V. cholerae*
p.	1	Simulated (30X)	Campylobacter and Vibrio	*Campylobacter lari*	*Not performed*
q.	1	One FP "Campylobacter" and one FN "Campylobacter" were processed together at the central reference testing site and may be a result of a sample mix-up.			
r.	1	One TP "Salmonella" and one FN "Salmonella" were processed together at the study testing site and may be a result of a sample mix-up.			

Table 7: Summary of Clinical Test Performance (n=1940) Compared to Broth Enrichment/EHEC EIA (***STX Combined***)

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
STX Combined	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 7/7 (59.0-100)	99.7% 1283/1287 [a,e] (99.2-99.9)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 13/13 (75.3-100)	99.5% 189/190 [b] (97.1-100)
	Simulated			409	98.2% 107/109 [d] (93.5-99.8)	98.7% 296/300 [c,f] (96.6-99.6)

	No.	Fresh, Frozen or Simulated (xLoD)	Identified by EP test as:	Identified by Reference/Comparator Method(s) as:	PCR Amp/BD Sequencing Results (if applicable)
a.	1	Fresh	Shiga Toxin 1	Negative	*Positive for Stx 1 gene*
	2	Fresh	Shiga Toxin 1 and Norovirus	*Escherichia coli*	*Positive for Stx 1 gene*
	3	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
b.	1	Select	Shiga Toxin 1 and *Campylobacter*	*Campylobacter*	*Positive for Stx 1 gene*
c.	1	Simulated (13X)	Shiga Toxin 1and *Salmonella*	*Salmonella enterica subsp. enterica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (30X)	Shiga Toxin 1and *Campylobacter*	*Campylobacter jejuni subsp. jejuni*	*Not performed*
	3	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*
d.	1	Simulated (30X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
	2	Simulated (2X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
e.	1	Fresh	Shiga Toxin 2	Negative	*Positive for Stx 2 gene*
	2	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
f.	1	Simulated (31X)	Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*

Table 8: Summary of Clinical Test Performance (n=1940) Compared to Broth Enrichment/EHEC EIA and Stx 1 and STX 2 typing

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
Stx1	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 4/4 (39.8-100)	99.8% 1287/1290[a] (99.3-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 9/9 (66.4-100)	99.5% 193/194[b] (97.2-100)
	Simulated			409	100% 50/50 (92.9-100)	99.2% 356/359[c] (97.6-99.8)
Stx2	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 6/6 (54.1-100)	99.8% 1286/1288[e] (99.4-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 10/10 (69.2-100)	100% 193/193 (98.1-100)
	Simulated			409	96.6% 57/59[d] (88.3-99.6)	99.4% 348/350[f] (98.0-99.9)

	No.	Fresh, Frozen or Simulated (xLoD)	Identified by EP test as:	Identified by Reference/Comparator Method(s) as:	PCR Amp/BD Sequencing Results (if applicable)
a.	1	Fresh	Shiga Toxin 1	Negative	*Positive for Stx 1 gene*
	2	Fresh	Shiga Toxin 1 and Norovirus	*Escherichia coli*	*Positive for Stx 1 gene*
	3	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
b.	1	Select	Shiga Toxin 1 and *Campylobacter*	*Campylobacter*	*Positive for Stx 1 gene*
c.	1	Simulated (13X)	Shiga Toxin 1and *Salmonella*	*Salmonella enterica subsp. enterica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (30X)	Shiga Toxin 1 and *Campylobacter*	*Campylobacter jejuni subsp. jejuni*	*Not performed*
	3	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*
d.	1	Simulated (30X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
	2	Simulated (2X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
e.	1	Fresh	Shiga Toxin 2	Negative	*Positive for Stx 2 gene*
	2	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
f.	1	Simulated (31X)	Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*

Table 9: Summary of Viral Target Clinical Test Performance (n=1940) Compared to Reference Methods (real-time RT-PCR and two conventional PCRs with bi-directional sequencing)

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
Norovirus	Clinical Specimens	Prospectively Collected	Fresh	1294	94.9% 37/39[a] (82.7-99.4)	99.6% 1250/1255[d] (99.0-99.9)
			Frozen	34	0% 0/1[b] (0.0-97.5)	100% 33/33 (89.7-100)
		Selected		203	100% 18/18 (81.5-100)	99.5% 184/185[c] (97.0-100)
	Simulated			409	-	100% 409/409 (99.1-100)
Rotavirus	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3[e] (9.4-99.2)	99.9% 1290/1291[g] (99.6-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	98.0% 50/51[f] (89.6-100)	100% 152/152 (97.6-100)
	Simulated			409	-	100% 409-409 (99.1-100)

	No.	SID	Specimen Type	EP test result	Composite Comparator Method Result*:
a	1	0611271	Fresh	Not Detected	Norovirus GII
	2	0611381	Fresh	Not Detected	Norovirus GII
b	1	065602	Frozen	Not Detected	Norovirus GI
c	1	973241	Selected	Rotavirus, Norovirus	Rotavirus,
d	1	052401	Fresh	Norovirus	Negative
	2	0610451	Fresh	Norovirus	Negative
	3	0611191	Fresh	Norovirus	Negative
	4	066181	Fresh	Norovirus	Negative
	5	069051	Fresh	Norovirus Shiga Toxin 1,	Stx1
e	1	067641	Fresh	Not Detected	Rotavirus
f	1	970381	Selected	Not Detected	Rotavirus
g	1	067631	Fresh	Rotavirus	Negative

*Reference method positive for Norovirus and Rotavirus: RT- PCR (endpoint PCR) with subsequent bi-directional sequencing must be positive.

Table 10: Summary of Genus/Group-Level Test Performance Versus Reference Method(s) – Stratified by Species

Campylobacter Genus				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Campylobacter	**91.7% 22/24 (72.0-99.0)**	**97.5% 39/40 (86.8-99.9)**	**98.5% 67/68 (86.3-100)**	**100% 15/15 (78.2-100)**
Campylobacter coli	100% 3/3 (29.2-100)	100% 3/3 (29.2-100)	100% 18/18 (81.5-100)	100% 5/5 (47.8-100)
Campylobacter jejuni	100% 1/1 (2.5-100)	97.3% 36/37 (85.8-99.9)	100% 9/9 (66.4-100)	100% 4/4 (40.0-100)
Campylobacter jejuni subsp. doylei	N/A	N/A	100% 5/5 (47.8-100)	100% 1/1 (2.5-100)
Campylobacter jejuni subsp. jejuni	90.0% 18/20 (68.3-99.8)	N/A	100% 21/21 (83.9-100)	N/A
Campylobacter lari	N/A	N/A	93.3% 14/15 (68.1-99.8)	100% 5/5 (47.8-100)

Shigella Genus				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Shigella	**66.7% 2/3 (9.4-99.2)**	**100% 8/8 (54.1-100)**	**100% 50/50 (92.9-100)**	**100% 20/20 (83.2-100)**
Shigella boydii	N/A	100% 2/2 (15.8-100)	100% 14/14 (76.8-100)	100% 5/5 (47.8-100)
Shigella dysenteriae	N/A	N/A	100% 9/9 (66.4-100)	100% 5/5 (47.8-100)
Shigella flexneri	N/A	N/A	100% 16/16 (79.4-100)	100% 5/5 (47.8-100)
Shigella sonnei	100% 2/2 (15.8-100)	N/A	100% 11/11 (71.5-100)	100% 5/5 (47.8-100)
Shigella spp not identified	0% 0/1 (0-97.5)	100% 6/6 (54.1-100)	*N/A*	*N/A*

Salmonella Genus				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Salmonella	**87.0% 20/23 (66.4-97.2)**	**98.3% 58/59 (90.9-100)**	**100% 67/67 (94.6-100)**	**100% 31/31 (88.8-100)**
Salmonella "non-typhi"	N/A	N/A	100% 2/2 (15.8-100)	N/A
Salmonella bongori	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)
Salmonella enterica	N/A	98.3% 58/59 (90.9-100)	100% 1/1 (2.5-100)	N/A
Salmonella enterica subsp. arizonae	N/A	N/A	N/A	100% 1/1 (2.5-100)
Salmonella enterica subsp. diarizonae	N/A	N/A	100% 3/3 (29.2-100)	100% 1/1 (2.5-100)
Salmonella enterica subsp. enterica	N/A	N/A	100% 52/52 (93.2-100)	100% 25/25 (86.3-100)
Salmonella enterica subsp. houtenae	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)
Salmonella enterica subsp. indica	N/A	N/A	100% 3/3 (29.2-100)	100% 1/1 (2.5-100)
Salmonella enterica subsp. salamae	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)
Salmonella spp not identified	87.0% 20/23 (66.4-97.2)	N/A	*N/A*	N/A

Vibrio Genus				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Vibrio	**100% 2/2 (15.8-100)**	**100% 1/1 (2.5-100)**	**91.1% 51/56 (80.4-97.0))**	**100% 10/10 (69.2-100)**
Vibrio cholerae	N/A	N/A	84.2% 16/19 (60.4-96.6)	100% 5/5 (47.8-100)
Vibrio parahaemolyticus	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)	94.6% 35/37 (81.8-99.3)	100% 5/5 (47.8-100)

Table 11: Clinical Mixed Specimen Combinations Detected by **EP**

Multiple Target Combinations Detected by EP			***Reference Test***		
Target 1	*Target 2*	*Target 3*	*Total Specimens*	*Discrepant Specimens*	*Discrepant Identification*
Y. enterocolitica	*Shiga Toxin 1*	*Shiga Toxin 2*	1	1	Stx 1 gene, Stx 2 gene
Campylobacter	*Shiga Toxin 1*	N/A	2	2	*Stx 1 gene*
Campylobacter	*Salmonella*	N/A	2	1	*Salmonella*
				1	*Campylobacter*
Campylobacter	*Vibrio*	N/A	1	1	*Vibrio*
Salmonella	*Shiga Toxin 1*	N/A	1	1	*Stx 1 gene*
Shigella	*Shiga Toxin 1*	N/A	1	1	*Shigella*
Y. enterocolitica	*Shiga Toxin 2*	N/A	1	1	*Stx 2 gene*
Shiga Toxin 1	*Shiga Toxin 2*	N/A	10	1	*Stx 1 gene* *Stx 2 gene*
Salmonella	*Norovirus*	N/A	2	0	*N/A*
Shiga Toxin 1	*Norovirus*	N/A	2	1	*Norovirus*
				1	*Stx 1 gene*
Norovirus	*Rotavirus*	N/A	2	1	*Norovirus*
TOTAL			***25***	***13***	

Table 12: Clinical Mixed Specimen Combinations Detected by Reference Methods

Multiple Target Combinations by Reference Test			***Detected by EP***		
Target 1	*Target 2*	*Target 3*	*Total Specimens*	*Discrepant Specimens*	*Discrepant Targets*
Shiga Toxin 1	*Shiga Toxin 2*	N/A	9	0	N/A
Salmonella	*Norovirus*	N/A	2	0	N/A
TOTAL			***11***	***0***	

B. Precision and Reproducibility

The ***Precision Study*** involved the daily testing of twenty (20) unique samples in duplicate by two (2) operators for four (4) non-consecutive days for a total of sixteen (16) tests per sample. In total, the study yielded 320 test results. The twenty (20) member sample panel, comprising representative strains of each of the nine (9) organisms/targets detected by **EP**, was prepared at two (2) different concentrations (18 positive samples; two Norovirus strains tested) and two (2) negative samples consisting of Negative Stool Matrix and *Clostridium difficile.* This panel included for each strain, a "Low Positive" sample, which would be expected to produce a positive result approximately 95% of the time, and a "Moderate Positive" sample, which would be expected to produce a positive result approximately 99% of the time.

The results of the precision study are summarized in **Table 13,** which provides the percent agreement between the expected results and the obtained results for each sample tested. The initial call rate was 98.1% (314/320). Of the six (6) initial No Calls, all 6 yielded a valid test result upon retesting for a final call rate of 100% (320/320). The Pre-Analysis Error rate was 0.9% (3/333). The final study results for the negative panel members, moderate positive samples and low positive samples agreed 99.7% with the expected results. One inaccurate call was made involving a *Salmonella enterica* specimen whereby **EP** unexpectedly detected "Stx2" in addition to "Salmonella". Additionally, acceptable precision was observed for the panel members across multiple consumable lots, days, operators, runs, instruments, and replicates.

The ***Reproducibility Study*** involved the testing of twenty (20) unique samples in triplicate by two (2) operators for five (5) non-consecutive days at three sites for a total of ninety (90) tests per sample. The twenty (20) member sample panel, comprising representative strains of each of the eight (8) organisms/targets detected by **EP**, was prepared at two (2) different concentrations (18 positive samples; two Norovirus strains tested) and two (2) negative samples consisting of Negative Stool Matrix and *Clostridium difficile.* This panel included for each strain, a "Low Positive" sample, which would be expected to produce a positive result approximately 95% of the time, and a "Moderate Positive" sample, which would be expected to yield a positive result approximately 99% of the time.

A total of 1800 initial tests were conducted. There were fourteen (14) Pre-Analysis Errors; these tests were repeated and valid test results were obtained for a PAE rate of 0.8% (14/1863). There were 49 initial No Calls, which were repeated once. All except six (6) of these repeats generated a valid result, yielding a final call rate for the study (number of valid tests/total tests conducted) of 99.7% (1794/1800).

The percent detection rate of the **Reproducibility Study** across all sites was 98.1%.There were 34 discordant calls (observed versus expected). Twenty-six (26) of the 34 discordant results were observed with the low positive samples, as expected. For the moderate positive samples, it is expected that the target(s) present in the sample will be detected approximately 99% of the time; in this study, the targets were detected at an acceptable rate of 99.0%. For the low positive samples, it is expected that the target(s) present in the sample will be detected approximately 95% of the time; in this study, the targets were detected at an acceptable rate of 96.8%. The final study results for the negative panel members demonstrated 100% agreement with the expected results. The results of the **Reproducibility Study** are summarized in **Table 14.**

Table 13: EP Precision Performance Study Summary

No.	Sample	Expected Call	Concentration	Call Rate (95 % CI)		Total Agreement with Expected Result (95 % CI)
				Initial	Final	
1	*Escherichia coli/Stx2*	Shiga Toxin 2 Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
2	*Salmonella enterica*	Salmonella Detected	Moderate	93.8% 15/16 (69.8%-99.8%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	93.8% 15/16 (69.8%-99.8%)	100% 16/16 (79.4%-100%)	93.8% 15/16 (69.8%-99.8%)
3	*Shigella dysenteriae/Stx1*	Shigella Detected Shiga Toxin 1 Detected	Moderate	16/16 100% (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
4	*Yersinia enterocolitica*	Yersina enterocolitica Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
5	*Campylobacter jejuni*	Campylobacter Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
6	*Vibrio parahemolyticus*	Vibrio spp. Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	87.5% 14/16 (61.7-98.5%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
7	*Norovirus GI*	Norovirus Detected	Moderate	93.8% 15/16 (69.8%-99.8%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	93.8% 15/16 (69.8%-99.8%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
8	*Norovirus GII*	Norovirus Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
9	*Rotavirus*	Rotavirus Detected	Moderate	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
			Low	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
10	Negative Stool Matrix	All Targets Not Detected	NA	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)
11	*Clostridium difficile*	All Targets Not Detected	NA	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)	100% 16/16 (79.4%-100%)

Table 14: EP Reproducibility Performance Study Summary

No.	Sample	Expected Call	Concentration	Call Rate (95 % CI)		Total Agreement with Expected Result (95 % CI)
				Initial	Final	
1	*Escherichia coli/Stx2*	Shiga Toxin 2 Detected	*Moderate*	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
			Low	88/90 97.8% (92.2-99.7)	90/90 100% (96.0-100)	89/90 98.9% (94.0-100)
2	*Salmonella enterica*	Salmonella Detected	*Moderate*	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	88/90 97.8% (92.2-99.7)
			Low	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	86/90 95.6% (89.0-98.8))
3	*Shigella dysenteriae/Stx1*	Shigella Detected Shiga Toxin 1 Detected	*Moderate*	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	88/90 97.8% (92.2-99.7)
			Low	88/90 97.8% (92.2-99.7)	90/90 100% (96.0-100)	86/90 95.6% (89.0-98.8)
4	*Yersinia enterocolitica*	Yersinia enterocolitica Detected	*Moderate*	90/90 100% (96.0-100)	90/90 100% (96.0-100)	89/90 98.9% (94.0-100)
			Low	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	80/90 88.9% (80.5-94.5)
5	*Campylobacter jejuni*	Campylobacter Detected	*Moderate*	88/90 97.8% (92.2-99.7)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
			Low	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
6	*Vibrio parahemolyticus*	Vibrio Detected	*Moderate*	87/90 96.7% (90.6-99.3)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
			Low	88/90 97.8% (92.2-99.7)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
7	Norovirus GI	Norovirus Detected	*Moderate*	87/90 96.7% (90.6-99.3)	88/90 97.8% (92.2-99.7)	86/88 97.7% (92.0-99.7)
			Low	87/90 96.7% (90.6-99.3)	90/90 100% (96.0-100)	86/90 95.6% (89.0-98.8)
8	Norovirus GII	Norovirus Detected	*Moderate*	81/90 90% (81.9-95.3)	87/90 96.7% (90.6-99.3)	86/87 98.9% (93.8-100)
			Low	83/90 92.2% (84.6-96.8)	89/90 98.9% (94.0-100)	89/89 100% (95.9-100)
9	Rotavirus	Rotavirus Detected	*Moderate*	89/90 98.9% (94.0-100)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
			Low	88/90 97.8% (92.2-99.7)	90/90 100% (96.0-100)	87/90 96.7% (90.6-99.3)
10	Negative Stool Matrix	Negative	NA	87/90 96.7% (90.6-99.3)	90/90 100% (96.0-100)	90/90 100% (96.0-100)
11	*Clostridium difficile*			86/90 95.6% (89.0-98.8)	90/90 100% (96.0-100)	90/90 100% (96.0-100))

C. Analytical Sensitivity (Limit of Detection)

Analytical sensitivity (LoD) of the **EP** was determined for 20 strains of Enteric Pathogens, representing all nine **EP** reportable target analytes. The LoD was defined as the concentration at which the test produces a positive result greater than 95% of the time. Serial dilutions of the strains were tested and the putative LoD confirmed with 20 replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further 20 replicates were performed at the next lower concentration until <95% was achieved. The LoDs for the 18 strains tested, and the corresponding LoD ranges for the **EP** reportable target, are shown in **Table 15** below.

Table 15: EP Limit of Detection (LoD units *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Y. enterocolitica* and *E. coli* in CFU/mL; LoD units for Norovirus is copies/mL; LoD units for Rotavirus is $TCID_{50}$/mL)

Representative Organism Tested	*Strain Number*	*Organism LoD*	*Reportable Target*	*Target LoD*
Campylobacter jejuni subsp *jejuni*	ATCC 43429	$3.70x10^4$	Campylobacter	$3.70x10^4$ - $1.11x10^5$
Campylobacter coli	ATCC 43482	$1.11x10^5$		
Campylobacter lari	ATCC 35222	$3.70x10^4$		
Salmonella enterica subsp *enterica* serovar Typhi	ATCC 9993	$3.33x10^5$	Salmonella	$3.33x10^5$
Salmonella enterica subsp *arizonae*	ATCC 13314	$3.33x10^5$		
Shigella dysenteriae / Shiga Toxin 1	ATCC 29026	$3.70x10^4$	Shigella Shiga Toxin 1	$3.70x10^4$ - $1.11x10^5$
Shigella flexneri	ATCC 25929	$1.11x10^5$	Shigella	
Shigella sonnei	ATCC 29030	$3.70x10^4$		
Shigella boydii	ATCC 12035	$1.11x10^5$		
Vibrio cholera	ATCC 39315	$1.11x10^5$	Vibrio	$3.70x10^4$ - $1.11x10^5$
Vibrio parahaemolyticus	ATCC 49398	$3.70x10^4$		
Yersinia enterocolitica	ATCC 700822	$3.33x10^5$	Yersinia enterocolitica	$1.11x10^5$ -$3.33x10^5$
	ATCC 23715	$1.11x10^5$		
E. coli – Shiga Toxin 1	ATCC 43890	$4.10x10^3$	Shiga Toxin 1	$4.10x10^3$ - $3.70x10^4$
E. coli – Shiga Toxin 2	ATCC BAA-176	$1.11x10^5$	Shiga Toxin 2	$3.70x10^4$ - $1.11x10^5$
E. coli – Shiga Toxin 1 / Shiga Toxin 2	ATCC 43895	$3.70x10^4$	Shiga Toxin 1 Shiga Toxin 2	
Norovirus GI	CDC 2142	$4.12x10^5$	Norovirus	$4.12x10^5$
Norovirus GII	ATCC D17219	$1.67x10^6$		$1.67x10^6$
Rotavirus Group A	ATCC VR-2550	$1.11x10^3$	Rotavirus	$1.11x10^3$
	ATCC VR-2551	$3.70x10^2$		$3.70x10^2$

D. Analytical Reactivity (Inclusivity)

Analytical reactivity of **EP** was demonstrated with a comprehensive panel of one hundred and eleven (111) clinically relevant bacterial strains and forty-one (41) clinically relevant viral strains representing temporal, geographical, and phylogenic diversity for each claimed target (**Table 16**). For the Stx1 and Stx2 targets, Shiga toxin producing organisms tested included the vast majority of serotypes isolated in the U.S and those that are outbreak-related. All 111 bacterial strains generated the expected result when tested in triplicate at a concentration of three times LoD. The majority of viral strains were also detected at three times LoD. The only exceptions were a few Norovirus GII strains that required concentrations between 10x and 50x LoD for 100% detection. Considering the *in silico* analysis, **EP** is expected to detect most of the Norovirus GII strains. Nevertheless, noroviruses are extremely diverse genetically, and detection of some strains may occur with reduced sensitivity.

Norovirus strains GII.9, GII.14, and GIV.1 and Rotavirus A strains G4, G5, G10, G11, and G15 are predicted to be detected based on *in silico* analysis. Norovirus GII.11 is not expected to be detected by EP. Additionally, based on *in silico* analysis, rare Norovirus genotypes GII.6 and GII.13 are predicted to either be not detected by EP or to be detected with reduced sensitivity. Inclusivity to Norovirus strains GII.8 and Rotavirus G7, G21, and G24 could not be evaluated due to a lack of available sequences.

Table 16: Organisms Tested for Inclusivity

Target Type	***Reportable Target***	***Total Number of Organisms/Strains Tested***	***Species/Genogroups Tested***		
			Name (Number of Strains)	*Total Number*	*LoD Concentration*
Bacterial	Campylobacter	15	*C. coli (5), C. jejuni subsp jejuni (4), C. jejuni subsp Doylei (1), C. lari (5)*	3	3x
	Salmonella	31	*S. bongori (1), S. enterica subsp various (5), S. enterica subsp enterica serovar various (25)*	2	3x
	Shigella	20	*S. boydii (5), S. dysenteriae (5)* [a], *S. flexneri (5), S. sonnei (5)*	4	3x
	Vibrio	10	*V. cholerae (5), V. parahaemolyticus (5)*	2	3x
	Yersinia enterocolitica	7	*Y. enterocolitica (7)*	1	3x
Toxin	Shiga toxin 1	19	*S. dysenteriae (2)* [a], *E. coli (17)* [b]	2	3x
	Shiga toxin 2	16	*E. coli (16)*[b]	1	3x
Viral	Norovirus	29	GI [13 including: GI.1, GI.2 (2 strains), GI.3 (2 strains), GI.4 (2 strains), GI.5, GI.6] and GII.3-1, GII.4-4, GII.5, GII.12-1]	2	3x
			GII.1, GII.2, GII.3-2, GII.3-3, GII.4-2, GII.4-3, GII.4-5, GII.10, GII.12-2	1	10x
			GII.7, GII.16, GII.17	1	50x
	Rotavirus	12	Group A [12 including G1 (4 strains) G2 (3 strains), G3, G6, G8, G9, G12]	1	3x

[a] Two (2) strains contain Shiga Toxin 1
[b] Five (5) strains contain both Shiga Toxin 1 and Shiga Toxin 2

E. Analytical Specificity (Exclusivity)

One-hundred and fifty-eight (158) organisms, consisting of 134 bacterial organisms, 18 viruses, four (4) parasites one (1) fungal organism and one (1) human cell line were tested with **EP** to determine analytical specificity (see **Table 17**). Eight (8) organisms, including Astrovirus and Sapovirus (2 strains), *Campylobacter hominis* and all four parasites were tested as genomic DNA/RNA. To rule out cross-reactivity between the analytes detected by **EP**, nine (9) organisms representing all of the **EP** detected targets, were tested at elevated concentrations of 5 x 10^6 CFU/mL for bacteria and at least 100x LoD for viruses. The exclusivity of the following was evaluated by *in silico* analysis only: 15 species of *Vibrio* not associated with human infection; four (4) non-pathogenic strains of *Escherichia coli*, *Yersinia pestis, Clostridium botulinum,* Norovirus genotypes/genogroups GIV.2, GIII, and GV, and Rotavirus genogroups B, C, D, and NADRV (with the exception of porcine strains within genogroup C).

All of the organisms tested yielded the expected "Not Detected" results, indicating that there was no cross-reactivity with **EP**, with the exception of *Campylobacter insulaenigrae* that yielded a single positive result (1/9) for "Campylobacter." *In silico* analysis also indicates a potential for low-level cross-reactivity. While *Campylobacter insulaenigrae* has been isolated primarily from marine mammals, in rare cases it may cause septicemia and gastroenteritis in humans.[19]

Table 17: Organisms Tested for Exclusivity

Bacteria				***Campylobacter, Vibrio, and Yersinia Species Not Detected by EP***	
Genus	***Species***	***Genus***	***Species***	***Genus***	***Species***
Abiotrophia	*defectiva*	*Escherichia*	*coli (3 strains)*	*Campylobacter*	*concisus*
Acinetobacter	*baumannii*		*coli* (EAEC)		*curvus*
	lwoffii		*coli* (EPEC) *(2 strains)*		*fetus*
Arcobacter	*butzleri*		*coli* (ETEC) *(2 strains)*		*gracilis*
	cryaerophilus		*fergusonii*		*hominis*
Aeromonas	*allosaccharophila*		*hermannii*		*hyointestinalis*
	bestiarum	*Fusobacterium*	*varium*		*insulaenigrae*
	caviae	*Helicobacter*	*hepaticus*		*lanienae*
	encheleia		*pylori (4 strains)*		*mucosalis*
	enteropelogenes	*Klebsiella*	*oxytoca*		*rectus*
	eucrenophila		*pneumoniae*		*showae*
	hydrophilia	*Lactobacillus*	*acidophilus*		*sputorum*
	jandaei		*reuteri*		*upsaliensis*
	*salmonicida**		*rhamnosus*	*Vibrio*	*alginolyticus*
	veronii	*Lactococcus*	*lactis*		*campbellii*
Alcaligenes	*faecalis*	*Leminorella*	*grimontii*		*cincinnatiensis*
Bacillus	*cereus*	*Listeria*	*grayi*		*fluvialis*
Bacteroides	*caccae*		*monocytogenes*		*furnissii*
	fragilis	*Morganella*	*morganii*		*harveyi*
	merdae	*Peptostreptococcus*	*anaerobius*		*metschnikovii*
	stercoris	*Plesiomonas*	*shigelloides*		*mimicus*
Cedecea	*davisae*	*Porphyromonas*	*asaccharolytica*		*tubiashii*
Citrobacter	*amalonaticus*	*Prevotella*	*melaninogenica*		*vulnificus* (3 strains)
	freundii	*Proteus*	*mirabilis*	*Yersinia*	*aldovae*
	sedlakii		*vulgaris*		*aleksiciae*
Clostridium	*bifermentans*		*penneri*		*bercovieri*
	bolteae	*Providencia*	*stuartii*		*frederiksenii*
	butyricum		*alcalifaciens*		*intermedia*
	difficile (2 strains)		*rettgeri*		*kristensenii*
	difficile, non-tox	*Pseudomonas*	*aeruginosa (2 strains)*		*mollaretii*
	haemolyticum		*fluorescens*		*pseudotuberculosis*
	methylpentosum		*putida*		*ruckeri*
	nexile	*Ruminococcus*	*bromii*		*rohdei*
	noyvi	*Serratia*	*liquefaciens*	***Viruses***	
	orbiscindens		*marcescens*	***Name***	***Serovar / Group***
	perfringens	*Staphylococcus*	*aureus*	Adenovirus	Type 1/Group C
	scindens		*epidermidis*		Type 2/Group C
	septicum	*Streptococcus*	*agalactiae, O90R*		Type 3/Group B1
	sordellii		*dysgalactiae*		Type 4/Group E
	spiroforme		*mutans*		Type 5/Group C
	sporogenes	***Parasites***			Type 14/Group B2
Collinsella	*aerofaciens*	*Blastocystis*	*hominis*		Type 26/Group D
Desulfovibrio	*piger*	*Cryptosporidium*	*parvum*		Type 31/Group A
Edwardsiella	*tarda*	*Entamoeba*	*histolytica*		Type 37/Group D
Enterobacter	*aerogenes*	*Giardia*	*lamblia*		Type 40/Group F
	cloacae	***Human Cell Line***			Human 4
Enterococcus	*faecalis*	Colon epithelial cells		Astrovirus	-
	faecium	***Fungal Strain***		Coxsackievirus B4	-
		Candida albicans		Cytomegalovirus	-
** Sub-species masoucida and sub-species salmonicida (2 strains)*				Echovirus 11	-
				Enterovirus 68	-
				Sapovirus (2 strains)	-

F. Microbial Interference

Two representative bacterial organisms detected by **EP**, *Campylobacter jejuni* and *Escherichia coli* (Shiga toxin 1), and two representative viral organisms, Norovirus GI and Rotavirus Group A, were evaluated for potential interference in the presence of fourteen (14) potentially interferent microorganisms not detected by **EP**: *Bacteroides fragilis, Prevotella oralis, Prevotella melaninogenica, Bifidobacterium bifidum, Clostridium perfringens, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Lactobacillus acidophilus, Staphylococcus aureus, Blastocystis hominis, Entamoeba histolytica,* and *Candida albicans*. These 14 microorganisms represent the most prevalent bacteria known to be present in the human colon and therefore the most likely to be encountered in stool specimens tested with **EP**. These normal flora organisms were tested at a concentration of 10^7 CFU/mL with the exception of the parasites *Blastocystis hominis* and *Entamoeba histolytica,* which were tested at $9x10^6$ cells/mL and $7x10^5$ cells/mL respectively. No interference was observed with **EP** for any of the samples tested.

G. Interference (Exogenous Substances)

A comprehensive interfering substances study was performed to assess the potential inhibitory effect of endogenous and exogenous substances that can commonly be found in clinical stool specimens. Four organisms representative of the target analytes detected by **EP**, i.e.; *Campylobacter jejuni*, *Escherichia coli* (Shiga toxin 1), Norovirus GI and Rotavirus Group A were individually challenged at 3x LoD with 22 potentially interfering substances (**Table 18**) at high, medically-relevant "worst-case" concentrations. None of the 22 substances tested showed any inhibitory effect on the detection of target enteric pathogens using **EP**.

Table 18: Exogenous Substances Evaluated for Interference

Intralipid (Triglyceride – Fecal Fat)	Options Conceptrol® Vaginal Contraceptive Gel	Pepto-Bismol Max Strength
Cholesterol (Fecal Fat)	Imodium® AD Anti-Diarrheal	Amoxicillin (Antibiotic)
Whole Blood	Wet Ones® Antibacterial Hand Wipes	Metronidazole Topical Cream (0.75%)
Mucus / Nasopharyngeal Swab Sample in UTM	K-Y® Personal Lubricant Jelly	Naproxen Sodium
Nystatin Suspension	Vaseline Original 100% Pure Petroleum Jelly	Barium Sulfate
Preparation H® Anti-Itch Hydrocortisone 1%	Tums® Antacid with Calcium Extra Strength 750	Mucin from bovine submaxillary glands, Type I-S (Dehydrated)
Desitin Maximum Strength Original Paste	Gaviscon® Extra Strength Liquid Antacid	
Preparation H® Hemorrhoidal Ointment	Mesalazine	

H. Carryover/Cross-Contamination Study

The potential for carryover and cross-contamination on the Verigene System was assessed by alternately testing nine representative high positive enteric pathogen samples (*Yersinia enterocolitica*, *Shigella dysenteriae* / Stx1, *Escherichia coli* / Stx2, *Salmonella enterica*, *Campylobacter jejuni* , and *Vibrio cholera* at $5x10^6$ CFU/mL, and Norovirus GI and GII and Rotavirus Group A at 100x LoD), followed by testing a negative stool sample. The high-titer sample was alternated with the negative sample three times on nine unique Verigene *SP* Processors. No carryover or cross-contamination was observed.

I. Competitive Inhibition

In order to assess competitive inhibition for **EP**, binary combinations of all test panel organisms representing all possible dual infections, were evaluated. Contrived samples were prepared in Negative Stool Matrix (NSM), with one panel organism present at a Low Positive titer (3x LoD) and a second organism present at a High Positive titer (> 10^6 CFU/mL stool). The performance of **EP** was evaluated with each of the 56 unique sample combinations tested in replicates of three (3). The results of the Competitive Inhibition testing are summarized in **Table 19**. No evidence of competitive inhibition was observed at the titers tested.

Table 19: Results from the Competitive Inhibition Study

Organism at low titer: 3x LoD	***Organisms at High Titer: 100x LoD***								***Total Detection Rate***	
	Campylobacter coli	*Salmonella enterica*	*Shigella dysenteriae (Shiga Toxin 1)*	*Vibrio Cholerae*	*Yersinia enterocolitica*	*Escherichia coli (Shiga Toxin 2)*	*Norovirus GI*	*Rotavirus*	***Tests***	***%***
Campylobacter coli		3	3	3	3	2[a, b] (6/6)	3	3	20/21 (26/27)[a, b]	95.2% (96.1%)
Salmonella enterica	3		3	3	3	3	3	3	21/21	100%
Shigella dysenteriae (Shiga Toxin 1)	3	3		3	3	3	3	3	21/21	100%
Vibrio Cholerae	3	3	3		3	3	3	3	21/21	100%
Yersinia enterocolitica	3	3	3	3		3	3	3	21/21	100%
Escherichia coli (Shiga Toxin 2)	3	3	3	3	3		3	3	21/21	100%
Norovirus GI	3	3	3	3	3	3		3	21/21	100%
Rotavirus	3	3	3	3	2[c] (6/6)	3	3		20/21 (26/27)[c]	95.2% (96.1%)

[a] For the Low Titer *Campylobacter coli* and High Titer *E. coli*, Stx2 sample, **EP** did not detect *Campylobacter* in one of the three replicates, although Shiga Toxin 2 was correctly identified in all cases. An additional 6 replicates were tested and the expected result was obtained for both analytes in all replicates.

[b] In one of three replicates, *Low titer organism* was not detected; *High titer organism* was correctly identified

[c] For the Low Titer *Rotavirus* and High Titer *Y. enterocolitica* combination, **EP** correctly identified both organisms in 2 of the 3 replicates and missed the detection of *Rotavirus* in one replicate An additional 6 replicates were tested and the expected result was obtained for both analytes in all replicates.

J. Cutoff Verification

Target mean intensity values observed with **EP** were examined for the testing of the sixteen (16) bacterial samples and three (3) viral samples used to establish the Limit of Detection of the assay. In addition, the cut-off data set included the test results of two (2) negative samples. With replicates of 20 for each sample and ten target spot groups evaluated per test, a total of 6160 data points (1320 expected positive) were assessed to verify the assay cut-off.

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TEST KIT LABELING

The contents of a Test Kit may use EN 980 graphical symbols. The symbols are defined below.

REF	Catalog number
	Use by YYYY-MM-DD
LOT	Batch code
SN	Serial number
	Manufacturer
	Upper Limit – Temperature limitation
	Upper and Lower Limit – Temperature limitation
	Consult instructions for use
KEY-CODE	Key-code; Use this key-code to obtain instructions for use at www.e-labeling.eu
	Harmful
	Flammable

PATENTS AND TRADEMARKS

The Verigene Reader may be protected by US patent 7,110,585 and other pending US and foreign patent applications. The Verigene Processor *SP* may be protected by US patents 7,396,677 and 7,625,746, and other pending US and foreign patent applications. The Verigene Test Cartridge and/or its method of use may be protected by one or more of the following US patents: 6,506,564; 6,602,669; 6,645,721; 6,673,548; 6,677,122; 6,720,147; 6,730,269; 6,750,016; 6,767,702; 6,759,199; 6,812,334; 6,818,753, 6,903,207; 6,962,786; 6,986,989; 7,321,829; 7,695,952; 7,773,790; 8,323,888**;** and other pending US and foreign patent applications.

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